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Impaired bacterial flora in human excluded colon

C NEUT, J F COLOMBEL, F GUILLEMOT, A CORTOT, P GOWER, P QUANDALLE, M RIBET, C ROMOND, AND J C PARIS

From Faculté de Pharmacie, Bactériologie, Lille, Faculté de Médecine, Clinique des Maladies de l'Appareil Digestif Lille, and Faculté de Médecine, Service de Chirurgie Adultes Ouest, Lille, France

SUMMARY **We compared the rectal microflora of 16 patients with surgically excluded colorectum with 16 healthy controls. The cause of diversion was inflammatory bowel disease (n=10), colon cancer (n=3), miscellaneous (n=3). Six patients had a diversion colitis. In the excluded colorectum, the total bacterial count was only slightly lower than controls but the variety of the flora was significantly reduced. This reduction was confined to strict anaerobes, mainly the genus *Eubacterium* and *Bifidobacterium*. Among aerobes, enterobacteria were more often isolated than in controls. This altered microflora of excluded colorectum could be involved in the mucosal damage observed in some cases.**

Human colon contains an unique microbial ecosystem composed by a large variety of bacteria mainly strict anaerobes.[1] The substrates for the microbial development are both exogenous (food residues not digested by human enzymes) and endogenous (such as bile and mucus).[1 2] Beneficial effects of the microflora on host are resistant to colonisation[3] and the formation of short chain fatty acids, substrates for the colonocytes.[4]

In patients undergoing colostomy the remaining colon and rectal segment are completely isolated from the upper gastrointestinal tract. Diversion of the faecal stream may be associated with diversion colitis.[5] Although, the pathophysiology of this disorder is unknown, the bacterial flora might be implicated either by implantation of harmful strains or by partial loss of the normal colonic microflora.[6] Only scarce information exists, however, about the bacterial population of excluded colons.[7] The aim of the present study was to determine the microflora of excluded colon in 16 patients operated on for various colonic disease.

Methods

PATIENTS

The clinical data of the 16 patients are summarised in Table 1, nine men, seven women ranging from 19 to 83 years (mean 46). Indication for colonic diversion was inflammatory bowel disease (IBD) in 10 patients (cases 7 to 16). The excluded bowel segment was sigmoid and rectum in 11/16 cases. All patients were asymptomatic at the time of the study and had received the same paraoperative antibiotherapy (metronidazole). The delay between surgery and bacteriological study ranged from 1·5 to 156 months (mean 27·5 months). Thirteen patients had colonoscopy at the time of the study. In eight of nine patients with IBD and three of four of the other patients the endoscopic appearance was that of a mild inflammatory colitis. Erythema and friability was present in 10 patients, inflammatory polyps in six and aphthous ulcerations in five. No inflammatory pseudomembranes were noted and in all cases the inflammation was predominant in the rectum. On biopsy, acute inflammation with focal oedema and lymphocytosis of the lamina propria was noted in eight cases, chronic inflammation with increased number and size of lymphoid nodules in two. Associated active colitis as evidenced by focal crypt abscesses or surface epithelial degeneration was present in two cases. No granulomas were seen.

CONTROL POPULATION

Sixteen healthy persons (not receiving any antibiotic for at least three months) aged from 20 to 50 years (mean age 27) had analysis of their rectal microflora.

Address for correspondence: Dr Christel Neut, Faculté de Pharmacie, Department Bacteriologie, F 59045 Lille Cédex, France.

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Table 1 *Clinical data in individual patients*

Case	*Age/sex*	*Diagnosis and indication for diversion*	*Type of diversion*	*Length of excluded colon*	*Delay from surgery to bacteriological study (mo)*	*Endoscopic findings*	*Biopsy findings*
1	63/M	Free perforation jejunal ulcer	Ileostomy	Entire colon rectum	2	Normal	AI
2	55/F	Sigmoid villous carcinoma	Ileostomy	Sigmoid rectum	2	Friability erythema inflammatory polyps	AI
3	75/M	Sigmoid diverticulitis with perforation	Colostomy	Sigmoid rectum	156	Friability erythema granularity	CI
4	63/F	Ischiorectal abscess	Colostomy	Sigmoid rectum	5	Friability erythema aphthous ulcerations inflammatory polyps	AI
5	59/M	Sigmoid adenocarcinoma	Colostomy	Sigmoid rectum	5	ND	ND
6	83/M	Sigmoid adenocarcinoma	Colostomy	Sigmoid rectum	1·5	ND	ND
7	43/M	Ulcerative colitis	Ileostomy	Sigmoid rectum	1·5	Erythema friability	ND
8	40/M	Ulcerative colitis	Colostomy	Sigmoid rectum	38	Friability erythema inflammatory polyps	Surf deg CI
9	35/M	Crohn's disease	Ileostomy	Sigmoid rectum	39	Erythèma friability aphthous ulcerations	Crypt abscess AI
10	30/F	Crohn's disease	Ileostomy	Sigmoid rectum	5	Aphthous ulcerations	ND
11	41/F	Crohn's disease	Ileostomy	Transverse and left colon sigmoid rectum	78	Erythema friability inflammatory polyps	AI
12	48/F	Crohn's disease	Ileostomy	Left colon sigmoid rectum	11	Friability erythema inflammatory polyps aphthous ulcerations	AI
13	31/M	Crohn's disease	Colostomy	Sigmoid rectum	26	ND	ND
14	19/M	Crohn's disease	Colostomy	Sigmoid rectum	12	Friability, erythema inflammatory polyps aphthous ulcerations	AI
15	32/F	Crohn's disease	Colostomy	Left colon sigmoid rectum	27	Friability erythema	ND
16	22/F	Crohn's disease	Ileostomy	Entire colon	31	Normal	AI

AI=Acute inflammation; CI=chronic inflammation; Surf Deg=surface epithelial cell degeneration; ND=not done.

SAMPLING

Swabs from the rectal mucosa and stomy (introduced to a depth of 5 cm into the stomy) were taken with Anerobic Culturette (Marion Scientific, Kansas City, USA) and processed immediately. A preweighed swab of the same composition allowed the determination of the retained weight.

BACTERIOLOGICAL ANALYSIS

All manipulations were made in anaerobic chamber (atmosphere N_2: 75%, H_2: 10%, CO_2: 15%). The swab was directly introduced into the first of the dilution series containing a reduced cysteinated ¼ strength Ringer solution.[8] Then further five 10-fold dilutions were made; 0·1 ml of each dilution were inoculated on two plates of non-selective blood agar (modified Columbia medium) and on plates of the same medium rendered selective by the addition of nalidixic acid (final concentration 40 µg/ml) or neomycin (final concentration 75 µg/ml). After heating for 10 minutes at 80°C, each dilution was also spread on a third plate of the non-selective medium (for the detection of germinating spore forms).

One series of plates with non-selective media was incubated for 48 h at 37°C under aerobic conditions, the other plates were incubated for one week at 37°C under anaerobic conditions. Then total counts were obtained for the different series and all different types of isolated colonies (at an average of 20 per sample) were subcultured and identified after their morphological and biochemical characteristics by established bacteriological criteria.[9-11] This type of bacteriological analysis recovered the quantitatively predominant bacteria (constituting generally more than 0·1% of the total flora).

STATISTICAL ANALYSIS

Student's t test or χ^2-test were used as required.

Results

BACTERIAL COUNTS

In excluded colons, total bacterial count, somewhat lower in the stomal sample, was similar to controls in the rectum (Table 2). Strict anaerobic bacteria were detected in 12/16 rectal samples and only six of 12

Table 2 *Bacterial counts in different samples in 16 patients with excluded colon and 16 controls*

	Excluded colon segment		*Controls*
	Rectal sample	*Stomal sample*	*Rectal sample*
	n=16	n=12	n=16
Total bacterial count (colony-forming units Log_{10}/g)	8·23 (1·09)	7·61 (2·05*)	8·88 (0·74)
Count of anaerobic bacteria (Log_{10}/g)	7·63 (1·19)* [12/16]	6·85 (2·48*) [6/12]†	8·52 (0·96) [16/16]
Count of aerobic bacteria (Log_{10}/g)	7·71 (1·16) [16/16]	7·41 (1·95) [12/12]	7·18 (1·53) [16/16]

Values are mean (SD); numbers in square brackets indicate the number of samples permitting detection of this type of bacteria. *Significantly reduced ($p<0·05$) *v* controls; †Significantly reduced ($p<0·01$) *v* controls.

Table 3 *Number of different species recovered from each sample in 16 patients with excluded colon and 16 controls*

	Excluded colon segment		*Controls*
	Rectal sample	*Stomal sample*	*Rectal sample*
	n=16	n=12	n=16
Number of different species			
Total	5·8 (2·8)*	4·8 (3·2)*	10·8 (3·3)
Strict anaerobes	2·9 (2·9)*	1·8 (2·6)*	8·8 (2·8)
Facultative anaerobes	3·0 (1·1)	2·8 (0·8)	2·0 (1·7)
Strict aerobes	ND	0·2 (0·4)	ND

Values are mean (SD); ND=not detected; *$p<0·001$ *v* controls.

stomal samples while they were detected in all rectal samples in controls. Their count (the sum of the count of the different species of anaerobes) was significantly reduced in the excluded colons in both rectal and stomal samples. On the contrary, facultative aerobes were present in all samples and their counts were similar in the excluded colons and in controls (Table 3).

VARIETY OF THE RECOVERED MICROFLORA

The number of recovered species was significantly reduced in rectal and stomal samples in excluded colons. This decrease was only the result of a lower variety of anaerobic bacteria when compared with controls (Table 3). Among the strict anaerobes mainly Gram positive non-sporulated rods like *Bifidobacterium* and *Eubacterium* were significantly reduced. For instance, the genus *Eubacterium* present in two of 16 rectal samples and one of 12 stomal samples in the patients was regularly (15/16 samples) detected in the control population (Table 4).

Table 4 *Number of samples containing strains belonging to the mentioned genera in different samples in 16 patients with excluded colon and 16 controls*

	Excluded colon segment		*Controls*
	Rectal sample	*Stomal sample*	*Rectal sample*
	n=16	n=12	n=16
Streptococcus	12	10	10
Staphylococcus	3	0	2
Lactobacillus	3	2	5
Enterobacteria	14	12*	9
Bifidobacterium	1‡	1†	12
Eubacterium	2‡	1‡	15
Bacteroides	8†	5†	15
Fusobacterium	3	1	2
Peptostreptococcus	8	2‡	14
Clostridium	4	3	10

*$p<0·05$; †$p<0·01$; ‡$p<0·001$ *v* controls.

Table 5 *Number of different strains recovered from samples in 16 patients with excluded colon and 16 controls*

	Excluded colon segment		*Controls*
	Rectal sample	*Stomal sample*	*Rectal sample*
Total number of strains	93	57	174
Total Aerobes	47†	35*	33
Streptococcus	12	10	10
Staphylococcus	3	0	2
Corynebacteria	1	0	2
Lactobacillus	3	2	5
Enterobacteria	28†	21†	13
E coli	12	9	8
Prot/Prov/Morg[1]	13	9	0
Others	3	3	5
Neisseria	0	0	1
Pseudomonas	0	2	0
Total Anaerobes	46†	22†	141
Gram positive rods	5†	2†	52
Bifidobacterium	1*	1	19
Eubacterium	2*	1†	31
Prop/actinom[2]	2	0	2
Gram negative rods	20	10	44
Bacteroides	16	9	42
Fragilis group	7	4	26
Others	9	5	16
Fusobacterium	4	1	2
Peptostreptococcus	15	4	28
Clostridium	6	6	16

*$p<0·01$; †$<0·001$ *v* controls. (1) Proteus, Providencia, Morganella; (2) Propionibacterium, Actinomyces.

The identification of all subcultured colonies recovered 93 different strains from the rectal samples in patients. Forty seven (50·5%) were aerobes and 28 (30·1%) belonged to the enterobacterial group (Table 5). In controls 174 strains from which 33 (19%) were

Table 6 *Number of strains recovered in IBD patients and six other patients*

	IBD	*Others*
Total number of strains	61	32
Total Aerobes	25*	22
Streptococcus	7	5
Staphylococcus	2	1
Corynebacteria	0	1
Lactobacillus	2	1
Enterobacteria	14	14
E coli	8	4
Prot/Prov/Morg(1)	6	7
Others	0	3
Total Anaerobes	36*	10
Gram positive rods	3	2
Bifidobacterium	0	1
Eubacterium	2	0
Propionibacterium	1	1
Gram negative rods	17	3
Bacteroides	13	3
Fragilis group	4	0
Others	9	0
Fusobacterium	4	0
Peptostreptococcus	14*	1
Clostridium	2	4

*$p<0.05$ *v* controls. (1) Proteus, Providencia, Morganella.

aerobes and only 13 (7·5%) enterobacteria were recovered. Organisms belonging to the genera *Proteus*, *Providencia*, and *Morganella* regularly found in excluded colons were completely lacking in controls.

No relationship was found between the bacterial counts or the variety of the recovered bacteria and the existence of a diversion colitis. Differences appeared between patients with IBD and the other patients. All rectal samples from IBD patients contained anaerobes while only two of six samples of the other patients were positive for these organisms. The mean value of different anaerobic species was higher in patients with IBD than in the other patients (3·5 *v* 1·7 species/sample). In IBD patients mainly species of the genera *Bacteroides* and *Peptostreptococcus* were found more frequently (Table 6).

Discussion

In this study, we have analysed the bacterial flora in 16 patients with excluded colon in a clinically stable situation and without antibiotics for at least six weeks. The main difference between healthy controls and patients was a quantitative and qualitative diminution of anaerobes. This decrease was present in rectal and stomal samples suggesting that the excluded colon contains a stable flora identical at its upper and lower end. The somewhat lower total bacterial count in the stomal sample might be caused by a higher oxygen exposure and a lower humidity at this site.

The modifications in excluded colon flora were found in both groups (IBD and non-IBD) of patients. A higher isolation rate of anaerobes, however, was observed in the IBD group. Despite the absence of endoscopic and/or histologic evidence for recurrence of Crohn's disease or ulcerative colitis in the excluded segments, this difference could be the result of underlying IBD. Indeed faecal flora has been reported as being enriched in anaerobes in Crohn's disease and ulcerative colitis.[12]

Bacteriological study of the excluded colon in our patients was in favour of a desequilibrated microflora which might no longer be able to exert its function of colonisation resistance. The frequent isolation of enterobacteria belonging to the genera *Proteus*, *Providencia*, and *Morganella* is in agreement with this observation. Nothing is known of the specific pathogenicity of these bacteria in the gastrointestinal tract. These types of bacteria, however, rarely isolated from healthy persons (they were absent in our controls) are more frequently recovered during gastrointestinal disorders and might represent an example of implantation of potentially harmful strains in excluded colon.[13]

Another consequence of the decrease in anaerobes could be a diminished production of short chain fatty acids: 14% of isolates from the rectal sample of patients in comparison with 26% in controls produced butyric acid *in vitro*. Butyric acid being the most important substrate for the colonocytes,[4] this finding might be relevant to the mucosal damage observed in some cases.[14] The recent report of the successful treatment of diversion colitis by washing the excluded segment with a solution containing a mixture of volatile fatty acids could favour this hypothesis.[15]

Finally, our results confirm the experimental data[16] showing that the excluded colon, deprived of exogenous substrates, might permit the multiplication of a large quantity and variety of bacterial species which are very different in their nutrient requirements: in our study 36 different species were isolated from the patients (49 in controls) such as predominantly saccharolytic species like *Lactobacillus* concomitantly to proteolytic species like *Peptostreptococcus magnus* or *P prevotii*. *Bacteroides* of the *fragilis* group, however (favoured in their growth by bile and predominant in healthy subjects) were not the most frequent Gram negative rods in our patients.

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